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USING DEWAR-FLASK CALORIMETRY AND RECTAL TEMPERATURES TO DETERMINE THE SPECIFIC ABSORPTION RATES OF SMALL RODENTS

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NOTICES

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

This report has been reviewed and is approved for publication.

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The Dewar-flask calorimetric method of determining SARs, as described by Blackman and Black ~~(2)~~ and Allen and Hurt ~~(1)~~, requires the use of two animal cadavers for each SAR determination. One cadaver is exposed to electromagnetic (EM) radiation and its average body temperature is calorimetrically determined; the body temperature of the other cadaver, known as the sham or control, is also calorimetrically determined. If the two animals are handled the same during the irradiation process and have similar masses, then the sham's body temperature is assumed to be the same as the preexposed non-sham cadaver. This assumption is necessary for determining the total change in temperature of the cadaver during irradiation (a specimen's SAR is easily computed when its resultant temperature change is known). We, however, have modified this procedure by assuming that the non-sham's preexposure rectal temperature is the same as its whole-body average temperature. This eliminates the need for the extra sham cadaver and streamlines the general procedures. Statistically, there is no significant difference between a cadaver's rectal temperature and its calorimetrically determined whole-body average temperature.

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USING DEWAR-FLASK CALORIMETRY AND RECTAL TEMPERATURES TO DETERMINE THE SPECIFIC ABSORPTION RATES OF SMALL RODENTS

INTRODUCTION

When doing research on the bioeffects of radiofrequency (RF) and microwave radiation, an important measure for quantifying the results of an experiment is the measure of a specimen's specific absorption rate (SAR). The SAR is expressed in watts/kilogram and indicates the energy absorption characteristics of a specimen exposed to electromagnetic (EM) radiation. Usually, the SAR is normalized to an incident power density of 1 mW/cm^2 at a specified frequency of EM radiation. In general, the value of the SAR is dependent on the specimen's chemical composition, mass, physical dimensions, and orientation in the EM field.

The Dewar-flask method of calorimetry is a relatively simple, straightforward way of determining the whole-body average SARs of small-bodied animals. We have used this method routinely for EM bioeffects studies of rodents in weight categories of up to 500 g. However, during our studies we made a few changes to our method of calorimetry.

The purpose of this paper is to present a modified method of Dewar-flask calorimetry and provide justification for its use. The usual procedure, as described by Blackman and Black [2] and Allen and Hurt [1], requires the use of two animal cadavers for each SAR determination. The modified method, by contrast, only uses one cadaver for each SAR determination.

The use of rectal temperatures in the modified method is the essential difference between these two methods, and eliminates the need for extra sham cadavers, and thus presents certain overall advantages.

THEORY

Before we discuss calorimetry, let us first present a generalized relation for determining a specimen's SAR. The SAR can be found using the following formula [3]: if we know how long the specimen was exposed to EM radiation and its resultant change in temperature then

$$SAR = 4186(C)(DT)/(Dt) \quad W/kg \tag{1}$$

where C = specific heat of the specimen [cal/g (^{o}C)], DT = the difference between the specimen's final temperature and its initial temperature (^{o}C), Dt = the total elapsed time of the irradiation in seconds. The equation does not account for heat loss during the period of irradiation. Of course, certain precautions or techniques are followed to minimize the losses. Although the formula seems simple, determining the change in temperature of the specimen is nontrivial. However, for our rodent cadavers, we use the technique of Dewar-flask calorimetry to determine the changes in temperature.

The calorimetric technique of determining a whole-body average temperature requires that the cadaver be immersed in a Dewar-flask containing a medium, such as water, at a known temperature; then, the temperature of the cadaver, following irradiation, can be determined by noting the final temperature of the cadaver/medium mixture. In the case of a rodent cadaver, it takes from 1 to 8 h for the cadaver and water to reach temperature equilibrium within the calorimeter (Dewar-flask). The immersion of the cadaver can only be done once. The calorimetry can be done only after the cadaver has been irradiated. Typically, to estimate the initial (i.e., preexposed) whole-body temperature of the exposed cadaver, calorimetry is conducted on a sham cadaver of similar weight. The whole-body temperature of the sham is then assumed to be the initial whole-body temperature of the irradiated cadaver [1,2]. This procedure requires, of course, two cadavers for each SAR determination, and with it additional errors corresponding to the use of the extra cadaver.

The modified procedure, that we've introduced, eliminates the need for the extra sham cadaver. We approximate the irradiated cadaver's initial whole-body temperature by acquiring its steady-state rectal temperature before irradiation. The rectal temperature is then used in the calculations assuming it to be the cadaver's initial body temperature. We are not saying here that the whole-body average temperature of a cadaver can be determined exactly by its rectal temperature. What we say, instead, is that by placing the cadaver in a stable temperature environment and after waiting for temperature equilibrium, then the cadaver's rectal temperature may be a close approximation to its average body temperature, at least, within statistical acceptability.

The calorimetric equation for determining the whole-body average temperature of a specimen immersed within a calorimeter is [1]:

$$Tc = Tf + (Mw + Zd)(Tf - Ti)/(Cc)(Mc) \quad {}^{\circ}C \tag{2}$$

where: Tc = average body temperature of specimen, at the time of insertion into calorimeter ($^{\circ}$C).

Mw = mass of water or medium (in grams) multiplied times its specific heat (cal/$^{\circ}$C).

Zd = heat capacity of calorimeter--determined experimentally using equation (2) with known quantities (cal/$^{\circ}$C)

Ti = initial water temperature of calorimeter, before immersion of specimen ($^{\circ}$C).

Cc = specific heat of cadaver or specimen--determined experimentally. [cal/g ($^{\circ}$C)].

Mc = mass of cadaver or specimen (g).

Once Tc is computed, the SAR of the cadaver can be calculated with equation (1). That is, DT = Tc - Tr; where Tr is the cadaver's rectal temperature before irradiation, and if the cadaver is a rat, we assume that its average specific heat is approximately .824 cal/g ($^{\circ}$C). The SAR is then easily computed with the rest of the known values.

MATERIALS AND EQUIPMENT

Our calorimeters are wide-mouth glass vacuum bottles surrounded by 3-in.-thick closed-cell rigid foam. Styrofoam lids are made to fit snugly over the top of the calorimeters. Each calorimeter can accommodate up to a 500 g-rat cadaver. All the calorimeters were tested for the ability to hold heat. Measured amounts of heated water were placed in each calorimeter. The calorimeters which consistently held their heat the longest were chosen for our studies.

To monitor the temperatures of the cadavers and calorimeters, we use flexible 35-cm-long thermistor type probes. The probes are connected to, and calibrated by, a BSD-200 medical thermometry system that records, prints, and graphs the relevant temperature data. The accuracy of our system is +/- .1 °C and the resolution is approximately .015 °C. The 1.1-mm thin lead non-metallic thermistor probes slide easily into 16-ga closed-ended plastic catheters. The catheters are used for inserting the probe leads into the cadavers before monitoring the rectal temperatures.

Foam containers, transparent to RF, were fabricated to hold the cadavers in place during irradiation. The containers help to reduce heat loss to the air during irradiation and handling.

PROCEDURE

The following procedures were generalized for use in determining the SARs of rodents or similar animals:

1. Kill the appropriate number of rats or mice.

2. Insert a temperature probe with plastic catheter at least 8 cm into the body of each cadaver through its rectum.

3. Place each cadaver into a foam container; place the containers into a temperature-controlled chamber. The temperature should be set near the irradiation chamber temperature to minimize the temperature differential between the rodents and the irradiation chamber.

4. Place 400 to 500 g of water into each calorimeter. The amount of water used depends on the requirement to adequately submerge the cadaver within the calorimeter.

5. Place the calorimeters in the same chamber as the cadavers or in another chamber with a similar temperature. Keep the lids closed to avoid evaporation.

6. Leave the cadavers in the chamber overnight or long enough to ensure equilibrium.

7. On the next morning, or after the rectal temperatures have stabilized, record the rectal temperatures for about 5 min before disturbing the chamber. Note: the rectal temperatures are usually different from the chamber temperature and different from each other. Be careful not to record the

rectal temperatures after the chamber has been disturbed. Our data suggests that these temperatures don't necessarily reflect the whole-body average temperatures of the cadavers.

8. Remove the rectal temperature probes from the cadavers and place the probes in the calorimeters to monitor and record the water temperatures.

9. Quickly move one of the cadavers with its foam container to the appropriate location for EM exposure.

10. After irradiation, quickly remove the cadaver from its holder and place it into the calorimeter. The cadaver should be placed in the calorimeter that has the same temperature probe which was used to monitor its rectal temperature; this will preserve the probe's relative accuracy.

11. Repeat steps 9 and 10 for each additional cadaver.

12. Keep the calorimeters in a temperature-controlled room or chamber so that the calorimeters' external temperature can be kept close to their internal water temperature, preferably to within a degree. Otherwise, there may be a significant heat exchange between the calorimeters and the environment. In this case, a correction technique, such as described by Blackman and Black [2], must be used to account for the temperature drift.

13. Monitor the calorimeter temperatures until equilibration is determined. Usually, equilibration takes from 1 h for a 100-g rat to more than 8 h for a 500-g rat.

Finally, after equilibration, the initial and final water temperatures can then be used to calculate a cadaver's whole-body average temperature using equation (2). The SAR can be calculated using equation (1).

JUSTIFICATION

In an effort to ascertain whether our modified method was accurate enough to replace the usual method of calorimetry, a comparison was made between a set of calorimetrically determined whole-body average temperatures and their respective steady-state rectal temperatures (Table 1). Over a period of 6 months, 19 Sprague Dawley male rat cadavers with masses ranging from 200 g to 400 g were used.

The procedures, materials, and equipment were essentially the same as given earlier. The main difference in procedures was that the transmitter was not turned on for irradiation. Other than this difference, the cadavers were handled exactly the same as if they were part of a SAR determination study.

A simple analysis of the results in Table 1 indicates that there is an average difference between the two sets of temperatures of -.008 $^{\circ}$C. The standard deviation was .15 $^{\circ}$C. Statistically, using a Student's t distribution, a test was done on the data at the .05 level of significance. The hypothesis tested for a difference between the two dependent means. The test showed that there was no significant difference between the two sets of temperatures. Qualitatively, the results suggest that the error attributable to the actual difference between a cadaver's whole-body temperature and its rectal temperature is probably negligible when compared to the other errors in the calorimetric procedure. For example, errors can be attributed to temperature probe inaccuracy, handling, and normal procedural variability. These errors would essentially be random and could account for the relatively large standard deviation (.15) of the data in Table 1. In any case, sufficient evidence has been provided to justify the use of the modified method of calorimetry.

In summary, there are three main advantages in using rectal temperatures over calorimetry of sham cadavers. First, the rectal temperatures require no calculations and are easier to obtain. Second, eliminating a sham cadaver reduces the error inherent in introducing two animals into the system (i.e., errors from using animals with different masses, different dimensions, different calorimeters, and from using different temperature probes). Finally, there is an increase in the number of data points available from a fixed set of animals. However, one disadvantage is that the rectal temperatures can only be used after a long waiting period for temperature equilibration.

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TABLE 1. RECTAL/CALORIMETRIC DATA COMPARISON

Mass* (g)	Rectal (°C)	Calorimetric (°C)	Difference (°C)
300	26.70	26.76	-0.06
300	26.54	26.59	-0.05
300	26.55	26.46	0.09
300	26.81	26.99	-0.18
300	26.69	26.58	0.11
300	26.82	26.60	0.22
200	26.53	26.48	0.05
400	26.22	26.35	-0.13
400	26.24	26.33	-0.09
400	26.11	26.38	-0.27
400	27.13	27.16	-0.03
400	27.14	27.41	-0.27
400	27.31	27.28	0.03
300	22.18	22.20	-0.02
300	22.04	22.16	-0.12
300	19.98	19.85	0.13
300	20.02	19.95	0.07
300	24.24	24.17	0.07
300	24.04	23.75	0.29

*Rounded to the nearest 100 g.

AVERAGE = -.008
STANDARD DEVIATION = .15
STANDARD ERROR = .034
N = 19